



The Influence of PH and Ionic Strength on The Concentration of α -Amylase Enzyme Using Electrofiltration

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Abstract

Enzyme is one of the important materials in the bioprocess industry. In order to get an enzyme as a product, a suitable separatory unit is required in the production process. This is because enzyme can be easily deactivated by heat, chemicals and physical treatments as well as hydrolyzed by other enzymes. Various techniques can be used in the separatory process, among others electrophoresis, chromatography, adsorption, crystallization, centrifugation and ultrafiltration. This research tried to investigate the influence of the pH at different values of 5,3; 7,1 and 9,1 also ionic strength using NaCl 5% w/v at a concentrated α -amylase using electrofiltration. The study was performed using an enzyme solution as a feed and water as a diluate into electrofiltration membrane that were connected to 60 volt power supply. From the experiment, it was found that the selectivity of water towards the enzyme's components increased with an increase in the concentration level (VCR). The highest selectivity; 31.27 was obtained at an enzyme solution with pH 5.3 with salt addition (5 % w/v NaCl). The highest selectivity obtained in the absence of salt was 14.62 at pH 7.1. An increase in a specific activity generally occurred at 2 times concentration further decreased at 5 times concentration for an enzyme solution using NaCl (5% w/v). The largest % rejection obtained was 71.12 at 5 times concentration of enzymatic solution with salt addition at pH 5.3.

Keywords: Amylase, ionic strength, pH, electrofiltration, selectivity.

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1. Introduction

The utilization of enzyme in industry is rapidly increasing. Currently, commercial enzymes have been widely used either by the food or non-food industry.

α -amylase enzyme can be produced by bacteria of the *Bacillus* genus, including *B. subtilis*, *B. licheniformis*, and *B. Stearothermophilus*. α -amylase enzyme can also be produced by the fungus from the genus *Aspergillus*, among others: *A. Niger* and *A. Oryzae*. Enzymes produced by bacteria called bacterial α -amylase and the enzyme produced by a fungus called fungal α -amylase.

α -amylase enzyme are used together with gluco amylase enzymes to convert tapioca into glucose. α -amylase enzyme are also need in the production process of bioethanol to convert the flour into sugar through the solving process of a complex sugar (Liquefaction).

In order to get enzymes as product, correct separatory units are needed in the production process. Enzymes can be purified and concentrated by chromatography methods, electrophoresis, adsorption, crystallization, centrifugation and ultrafiltration (Ghost, 2003).

Purification and concentration of enzymes using membranes are advantageous in terms of energy save and scale up ability as compared to the chromatography and electrophoresis (Ghost, 2000). Besides, such process is performed at a relatively lower temperature and pressure. This will minimize the denaturation effect on the enzyme. H. Szatajer and M. Bryjak (1983) reported the use of polyacrylnitrile (PAN) and polysulphone (PS) membranes for fractionation and concentration. The results showed that the PS membrane was more suitable for the concentration of enzymes while the PAN membrane was for early fractionation.

Zeeman and Zydney (2003) used 2 stages membranes to separate α -lactalbumin and β -lactoglobulin using 100 and 30 kDa membranes connected in series.

Anna person, Jonsson and Zacchi (2003) used nilon and poly ether sulphone (PES) based microfiltration membranes to separate BSA from a protein solution.

Enevodsen, Hansen and Johnson (2006) used cross flow electro-ultrafiltration membranes to improve the performance of ultrafiltration membranes during purification. This is because enzymes carry electricity load which enable the loaded molecules to be separated away from the membrane's surface by combining ultrafiltration membranes with DC (direct current) to avoid the polarization of concentration and fouling. The combination of ultrafiltration membrane with AC (alternating current) has also been reported (Zumbus ,

Kulcke and Bruneer, 1998). Firdaous et al. (2009) used electrodialysis with ultrafiltration membranes to concentrate and separate selectively from bioactive peptide from hydrolysed protein of alfalfa crops.

2. Basic Theory

α -amilase enzymes contain a group of amino acids containing carboxylate ($-\text{COO}^-$) and ammonium ($-\text{NH}_3^+$) chains. The simultaneous presences of both chains cause the enzyme to act as an amfother, able to react with acids and base simultaneously and producing cation and anion. This can cause the pH of the solution to determine the electric capacity of the enzyme. The value of pH where an enzyme does not have such capacity is called isoelectric point (pI). An enzyme will have a negative charge at a pH above the pI and have a positive charge below the pI. The difference in the enzyme's carriage will influence the mobility values as well as the diffusion coefficient. Taking into account that these two parameters determine the behaviour of the mass transfer, hence, the loading difference can be utilized to separate the enzyme based on electrical interactions between enzyme-membrane, enzyme-enzyme or enzyme-solvent.

Studies show that membrane filtration for protein and enzymes are highly influenced by the natural characteristics and interactions between solutes (physicochemical interactions). The interaction between membrane and solute can occur in a form of electricity loading, hydrophobic or even loading transfer. The advantage is that the transmission from the solute via the membrane can be manipulated through the concentration of α -amilase enzyme. Physicochemical interactions such as pH and ionic strength or other parameters such as transmembrane pressure configuration system (Zulkali, Ahmad and Derek, 2004).

Pujar and Zydney (1994) showed that the rejection coefficient of bovine serum albumin (BSA) could increase from 0.2 to more than 0.995 due to the low ionic strength. The increase in protein rejection is influenced by the electrostatic repulsion from the negative protein carrier derived from the membrane pores. Fane, Fell and Waters (1983) mentioned that during the isoelectric point, the present of salt can decrease the retention from the neutral protein. The hydrodynamic radius of a protein can be manipulated using salt. Pujar and Zydney (1994) reported that the influence of ionic strength can decreased protein transmission. The influence of pH and ionic strength can be so large and unlimited to the ultrafiltration membrane especially but also applied to microfiltration membrane.

The membrane used in this experiment is an electrodialysis. Since this is a porous membrane, it can

also be called as electrofiltration membrane. Electrodialysis process is a membrane based process where the ions move from one solution to another solution through an ionic exchanger membrane with electric potential as a driving force (Mulder, 1996). This process can only be used to separate or remove ionic molecules

One of main parameters that affect the efficiency of the electrofiltration process is the selectivity. If value of selectivity of an electrofiltration process is higher, the feasibility of electrofiltration process using the membrane will be better. Selectivity of a membrane is represented by a dimensionless parameter α (separation factor). Water selectivity of the enzyme is calculated by the following equation:

$$\alpha = \frac{\left(\frac{C_{\text{water}}}{C_{\text{enzyme}}} \right)_{\text{diluate}}}{\left(\frac{C_{\text{water}}}{C_{\text{enzyme}}} \right)_{\text{feed}}} \quad (1)$$

Other parameters that show the performance of electrophoresis is the rejection value of an enzymes components. Higher rejection value reflects a better performance. The rejection value can be obtained by comparing the concentration of a diluate with regard to the concentration of a concentrate formulated (feed solution) as follows:

$$R = \left(1 - \frac{C_{\text{diluate}}}{C_{\text{feed}}} \right) \times 100\% \quad (2)$$

3. Experimental

3.1 Materials

Materials used in this experiment are α -amilase enzyme obtained from PT. Raya Sugarindo Inti. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) are obtained from Laboratorium Proses Hilir PAU ITB. Materials/reagents used for the analysis of enzymatic concentration are Bradford Merk Fermentas, Bovine Serum Albumin (BSA) and aquadest. Materials used for the analysis of enzymatic selectivity are 1 M HCl, 0.125% starch solution and KI/ I₂ solution (2% KI in 0.2% I₂). Materials used for the analysis of enzymatic level and activity are obtained from Biochemistry laboratory FMIPA ITB.

3.2 Equipment

Equipments used in this experiment are 1 litre measuring glass, electrofiltration plate and frame module with 840cm² wide, membrane used in this experiment is an anion exchanger (MA-3475) and cation exchanger membrane (MC 3470) with the following specification in table 1

Equipments for the analysis of enzymatic level and activity comprises of Smartfac Merk Biorad spectrophotometer.

Table 1. Characteristics of The Ion Exchanger Membrane Ionac

Membrane	Kation, MC-3470	Anion, MA-3475
thickness, mls	15	16
total capacity, meq/ml	1,4	0,9
Mullen burst, min, psi	150	200
Resistance area (ohm/cm ²) 0,1 N NaCl 1,0 N NaCl	25 10	50 25
Permselektifitas 0,5 N NaCl / 1,0 N NaCl	96	99
Water Permeability (ml/jam/ft ² /psi)	15	20
Temperature stability, max °F °C	176 80	176 80
Current density, max (Amps/ft ²)	50	50

3.3 Sample Analysis

Bradford method was used to analyze the level of enzyme. As for the analysis of enzymatic activity, fuwa method was used.

3.4 Research Methodology

This research aims to study the possibility of electrofiltration for the concentration of α -amilase enzyme at different pH and ionic strength. Data obtained includes the concentration and activity of enzyme at the feed as well as diluate.

In order to get the desirable result, the research methodology involved equipment setting, preparation and measurement of feed solution and diluate, sampling

and product analysis. The experimental flow chart is shown in Figure 1

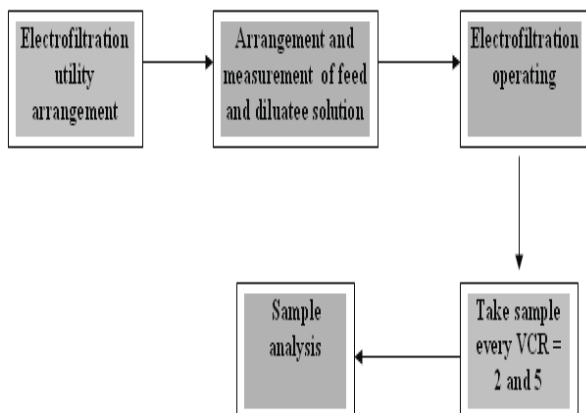


Figure 1. Flow Chart Experiment

The electrofiltration equipment was set as shown in figure 2. Preparation of enzyme solution and measurement of feed and diluatee solution. 500 ml of enzyme solution and diluatee (aquadest) were prepared. The pH was varied as 5,3; 7,1 and 9,1. The pH was controlled by adding HCl or NaOH. As for the weight of the ionic strength (NaCl salt 5% w/v – 25 gram in 500 ml enzyme solution) was used in digital mass balance.

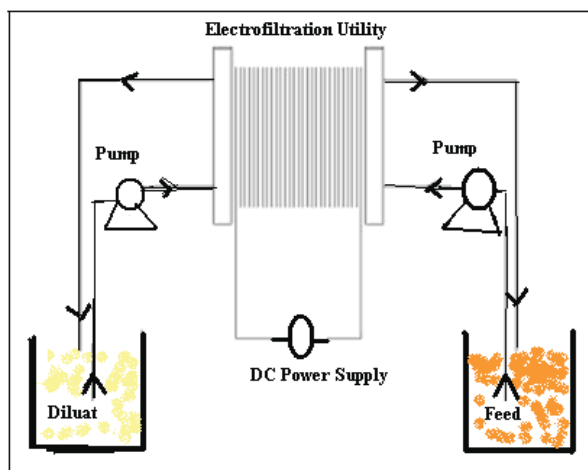


Figure 2. Electrofiltration Equipment

The electrofiltration was performed by controlling the voltage at 60 volt, the rate of diluate used was 150 ml/minute whereas the rate of the feed (enzyme solution) was set at 1200 ml/minute.

The initial concentration of enzyme used was 1 mg/ml with specific activity 107.119 U/mg protein. Sampling was performed for the feed (concentrate) and diluate, at the beginning of the operation and when VCR 2 and 5 times were achieved.

The samples were analyzed as previously mentioned. Then, the membrane was washed with RO water.

4. RESULTS AND DISCUSSION

4.1 The Influence of pH And Ionic Strength Towards Of The Membrane's Selectivity

The pH of the feed; 500 ml enzyme solution were arranged at 5,3; 7,1 and 9,1. The other feed was added with NaCl salt of 5% mass/volume. Then the diluate; 500 ml aquadest was prepared. Both the diluate and the feed solution were channelled into the electrofiltration membrane. Samples were taken after 2 and 5 times concentration. In preliminary experiments, the observations of volume transfer of the feed to diluate after lasting more than 5 hours there was relatively no significant changes in the same flow rates. Because electrofiltration use cation and anion membrane, where the ions move from one solution to another solution through ion exchange membrane with an electric potential as the driving force. Where the range pores of cation and anion membrane is 0.1 - 2 nanometers while the size of water molecules 0.278 nanometers. By seeing these two measures, the water molecules difficult through membrane pores.

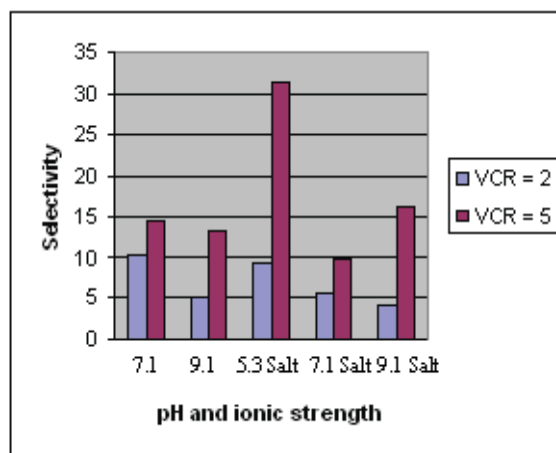


Figure 3. Selectivity in Various pH and Ionic Strength

To accelerate the transfer is given a different volume flow rates in feed large (1200 ml / min) and diluate (150 ml / min), with the hope of the water

molecules can be forced to penetrate the membrane pores of elektrodialysis.

The highest selectivity is pH 5.3 with salt addition 5% w/v. It can be explained, the concentration levels of enzymes that passes at pH 5.3 is smaller than pH 7.1 and 9.1 in diluate. A little concentration level of Enzyme pass in diluate due to the presence of salt will give an ionic strength on the enzyme that will cause conformational change and dispersion of the enzyme.

At the isoelectric point, without the presence of salt, protein molecules or enzymes in a compact form and has no net charge. The presence of ionic salt at this condition result increasing net charge and size of the enzyme. The increasing size of enzyme will reduce transfer rate of enzymes to diluate.

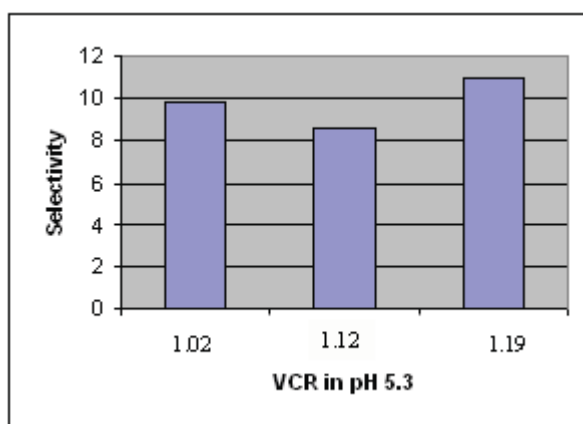


Figure 4. Water Selectivity Curve of the Enzyme at pH 5.3 without Ionic Strength

From figure 3 and 4 show that pH 7.1 and 9.1 without salt provide higher selectivity than pH 5.3. This can be explained at pH above Isoelectric point - Isoelectric point α -amylase enzyme which ranged from 4.7 to 5.2 (Yuhuan Wang, 1999 and Yoshikawa, 2000) - the enzyme has a load back so the increasing electrostatic repulsion interactions between enzyme molecules. The higher pH value will cause addition the number of sediment and particles salt which no dissolve making precipitated on the membrane surface. From the picture above, it can be seen trend that decreasing selectivity happened with the increasing pH.

Figure 4 presented observations of water selectivity of enzyme concentration on the value of 1.02; 1.12 and 1.19. 15 minutes were needed to obtain 5 times concentrated value (VCR=5) at pH 7.1 and 9.1. Whereas at pH 5.3, 250 minutes were needed to obtain a VCR value of 1.19 so that at this pH 2 and 5 time concentration requires a longer time as compared to the other 2 pH values. The value at pH 5.3 was closed to

the isoelectric point, the enzyme has approximately zero load that requires a longer time for mass transfer from the solution towards the diluate.

4.2 The influence of pH and ionic strength of the specific activity of protein

The effect of specific activity at various pH and salt addition 5 % w/v is presented in Figure 5. From the figure, it can be observed that there was an increase of specific activity from its initial value towards 2 times concentration (VCR = 2) then the specific activity decreased in the range of the 5 times concentration (VCR =5)

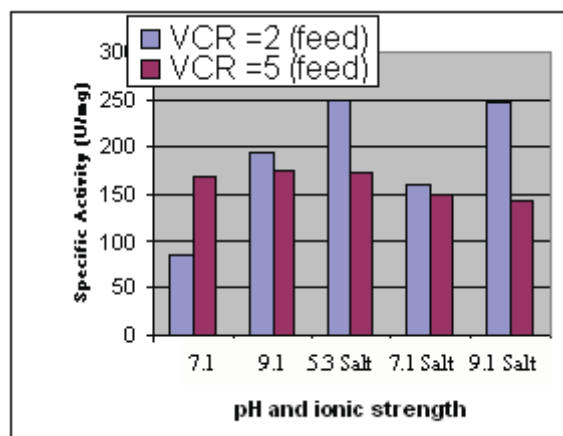


Figure 5. Specific Activity in Feed at Various pH and Ionic Strength

An increase of specific activity occurred at 2 times concentration. This was happening due to large amount of non-enzymatic protein or other ionic components that moved from the feed solution to the diluate so that the 2 times concentration had a positive correlation with the purity level of the enzyme.

At 5 times concentration, it could be observed that the specific activity decreased which potentially caused by 2 factors. First, denaturation of enzyme occurred that caused the deactivation of enzyme at 2 times concentration. Second, there were relatively large amount of enzymatic protein that migrated from the feed solution to the diluate. Hence, 2 times concentration is the optimum conditions for pH variation with the addition of salt.

Figure 6 also explain that pH 7.1 and pH 9.1 with salt addition obtained values of small specific activity. This result is good because only a little specific activity decrease in feed.

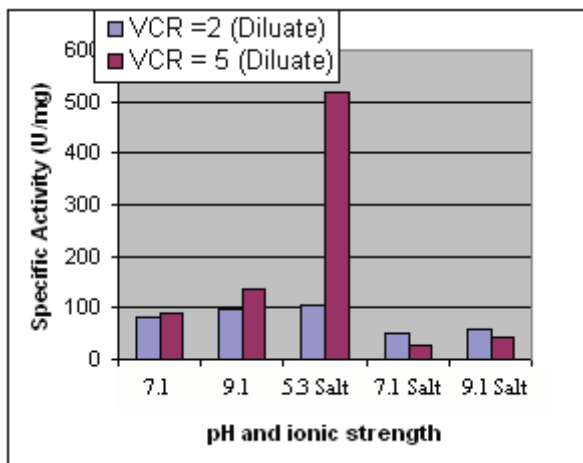


Figure 6. Specific Activity in Diluate at various pH and ionic strength

Figure 6 also explain that pH 7.1 and pH 9.1 with salt addition obtained values of small specific activity. This result is good because only a little specific activity decrease in feed.

The observations on diluate shows that the increasing specific activity occurred at pH 5.3 (VCR =5) with salt addition. At pH 5.3 with salt addition, the ions move from feed to diluate through ion exchange membrane with an electric potential as the driving force, in addition high flow rate of feed causing transfer volume of the feed solution to diluate. It makes much of enzymes pass the membrane pores. From the figure 6, it can be seen that the influence of flow rate is more dominant compared with the influence of salt addition which the salt addition increased the size of the enzyme.

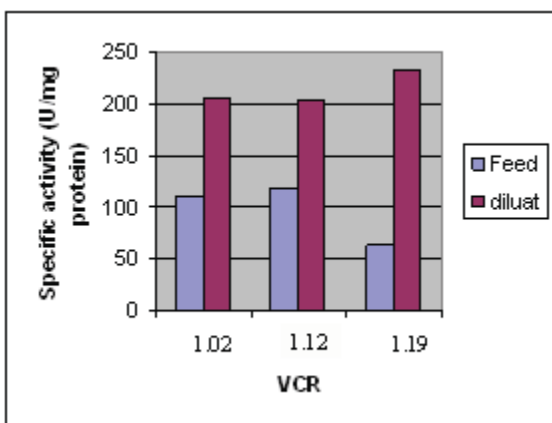


Figure 7. Influence curve pH 5.3 without salt addition

Figure 7 showed that pH 5.3 without salt addition, the specific activity of enzyme in diluate

increase after VCR more than 1.12. This shows an increasing of enzyme that passes through the membrane pores is proportional to the concentration level. To get 1.19 times concentration, 250 minutes was needed. This is different from the time needed to reach 5 times concentration at similar conditions.

A long duration process occurred at pH 5.3, in the absence of salt, the specific activity of α -amylase enzyme decreased throughout the concentration process due to the denaturation of the enzyme. The loss of enzymatic activity throughout a purification process had been widely. Whitaker (1972) reported that purification can cause the loss of essential cofactor which can result in the loss of enzymatic activity.

4.3 % Rejection value

Figure 8 illustrates that the rejection percentage at various pH and the influence of salt. The highest rejection results was obtained at pH 5.3 with the addition of salt at 5 times concentration. the greater rejection on the condition where feed solution has a pH close to isoelectric point. It cause the electric field does not affect the movement of ions enzyme move to diluate. However, by adding salt or without the addition of salt % the rejection falls with increasing pH at concentration 2 times it's because the enzyme has a charge so that electric fields affect the movement of ions in feed solution to diluate.

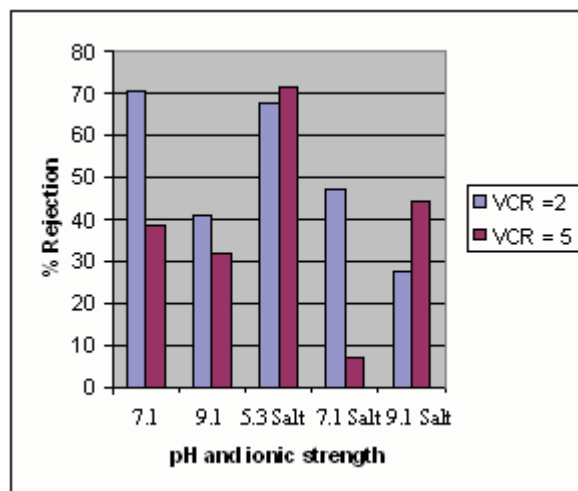


Figure 8. Curve % rejection at various pH

In general, this value indicated that there were still relatively large amount of enzyme components that pass through at 5 times concentration using electrofiltration. Relatively large amount of enzyme components that pass through because the electrofiltration only used ionic membrane exchanger from which the main function of this membrane is to

shift ionic components and prevents fouling but not to stop enzyme components using certain sizes as in the used of ultrafiltration. Hence, another one ultrafiltration membrane needs to be added to the electrofiltration configuration that functions to stop the enzyme components with certain molecule weight. Such configuration is known as electro-ultrafiltration.

5. CONCLUSIONS

From this research performed using electrofiltration for concentration of α -amilase enzyme with the variation of pH and ionic strength far from initial concentration condition, it can be concluded that:

The highest selectivity of water towards enzyme components occurred at the enzyme solution at pH 5.3 with the presence of NaCl (5% w/v) whereas for enzyme solution without the presence of salt occurred at pH 7.1. The highest % rejection occurred at pH 5.3 with salt addition. The highest specific activity of enzyme in feed occurred at pH 5.3 and 9.1 at VCR =2 with salt addition

The optimum conditions to concentrate α -amilase enzyme using electrofiltration membrane must consider the high specific activity of enzyme in feed and lower specific activity enzyme in diluate after concentrate then operation parameter of membrane likes selectivity and % rejection. From this research by considering all factor above, the optimum condition to concentrate α -amylase by electrofiltration is pH 5.3 with salt addition at VCR =2.

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